

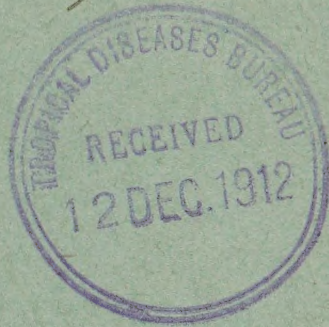
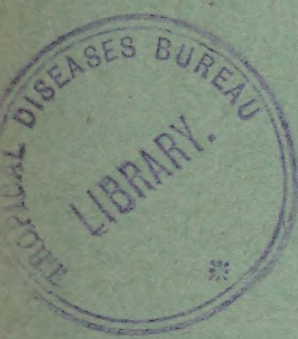
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STUDIES IN RELATION TO
MALARIA



BY

SAMUEL T. DARLING, M. D.
CHIEF OF LABORATORY



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Errata in pamphlet on STUDIES IN RELATION TO MALARIA
BY S. T. DARLING, M. D.

- Page 6; 24th line from bottom; Delete shown in the drawings.
Page 7; 28th line from bottom; Read coxae for cozae.
Page 8; 16th line from top; Read nape for nate.
Page 12; 8th line from top; Read anaerobic for aerobic.
Page 16; 3d line from bottom; Delete a
Page 23; 25th line from bottom; Read *malefactor*.
Page 26; 12th line from top; Read larval.
Page 26; 18th line from bottom; Read on the, for, with.
Page 27; 29th line from top; Read spermatheca for spermathecae.
Page 29; 8th line from top; Read than 38° Baume.
Page 29; 10th line from top; Read analysis for anaylses.
Page 30; 18th line from top; Read remains for remained.
Page 31; 21st line from bottom; Read passed for passes.
Page 33; 13th line from top; Read and in these are seen.
Page 33; Chart 51499; Read polymorphonuclear for ploymorphonucle
Page 34; 9th line from top; Delete as in the preceding one.
Page 35; 5th line from bottom; Read gametes in films.
Page 36; 3d line from top; Read forms.
Page 36; 21st line from bottom; Read 1 blood negative; entered
hospital few days later with tertian malaria.
Page 36; 20th line from bottom; Read tertian, all ages.
Page 37; 15th line from bottom; Read tertian, all ages.
Page 37; 19th line from top; Read became for become.
Page 38; 7th line from top; Read hemopoietic.
Page 38; 13th line from bottom; Read eosinophilia.
Page 38; After last line; Mr. A. H. Jennings for the indentification
of mosquitoes.

STUDIES IN RELATION TO MALARIA.

SAMUEL T. DARLING, M. D.,

Chief, Laboratory of the Board of Health.

The following observations and experiments have developed out of work directly or indirectly related to a study of some of the factors concerned in the transmission and prevention of malarial fever in the Canal Zone:

In every malarial region it is important that the varieties of mosquitoes common to that region should be recognized, their breeding habits should be studied, and a determination made of the species of anophelines hospitable to malaria, and those transmitting it. The English observers, James, Christophers, and Stephens, noticed that certain species of anophelines were natural transmitters of malarial fever, while others were rarely, if ever, found infected naturally, although it would be possible to infect them under laboratory conditions. We know that the breeding habits of anophelines vary, too, considerably, and it may be said that there is as much selection of breeding places by anophelines as there is selection of feeding grounds by fish. Trout, salmon, and bull-heads have their analogues among anopheles larvæ, some of the latter requiring fresh aerated water, or water containing much green algæ. Others are found in tree holes and the recesses of epiphytic tree plants, such as bromelias, where they prey upon other species; while others preferring fresh aerated water are so adaptable that they will flourish in sewage, streams, or in brackish water containing half its volume of sea water. Some species require an abundance of sunlight, while other sylvan species prefer shady pools in which chlorophyll-bearing algæ are relatively absent. The anophelines insusceptible to malaria may be more limited in their choice of breeding places, so that in the work of malarial-mosquito destruction the latter may be disregarded, and attention given wholly to the breeding places of those varieties responsible for the transmission of malarial fever.

With regard to man as a host, it is necessary to have some knowledge of the limits of his infectiousness, i. e., the number of sexual forms of the malarial parasite necessary to infect susceptible mosquitoes.

Besides the question of hospitable species of anophelines, there are other matters of much importance, such as latent malaria; the effect of quinine on the parasites in man; the value of various larvacides;

algacides; agents destructive to ditch grass, and a knowledge of the quality of wire screening, and the size mesh necessary in keeping out mosquitoes.

OUTLINE OF THE SUBJECTS CONSIDERED.

Anophelines of this region.
 Collection of larvæ.
 Breeding out mosquitoes and methods of feeding.
 Biting—infecting experiments.
 Estimation of gametes.
 Care of mosquitoes after feeding.
 Method of examining for zygotes and sporozoites.
 Description of the malarial parasite in the mosquito.
 Table of infecting experiments.
 Notes and conclusions from table of infecting experiments.
 Limit of infectiousness of man.
 Notes on the bionomics of anophelines.
 Effect of salt or sea water on larvæ.
 Experiments with larvacides.
 Experiments with agents destructive to vegetation, grass, and algæ.
 Experiments with screening of various mesh.
 Relative value of wire screening of various composition, based on practical tests and chemical analyses.
 Note on the value of the practice of killing anophelines found in quarters and barracks.
 Latent malaria.
 Effect of quinine upon the parasite in mosquito and man.
 The following is a list of anophelines of the Canal Zone:

Anopheles argyritarsis, R. D.
Anopheles tarsimaculata, Goeldi.
Anopheles gorgasi, D. K.
Anopheles albimanus, Wied.
Anopheles cruzii, D. K.
Anopheles apicmacula, D. K.
Anopheles punctimacula, D. K.
Anopheles malefactor, D. K.
Anopheles eiseni, Coquill.
Anopheles franciscanus, McCrack.
Anopheles pseudopunctipennis, Theob.

The above 11 species of anophelines have been collected in the Canal Zone during the past five years. They are not taken, nor do they exist in their breeding places, in anything like equal numbers. For example: Only one specimen of *A. gorgasi* has been found. Of the 11 species, the commonest ones are *A. albimanus*, *A. pseudopunctipennis*, and *A. malefactor*, but this again must be qualified by stating that the predominance of a species varies from season to season and from place to place. In certain villages, upon going through the barracks only, *A. albimanus* will be found, while in other villages from 5 to 10 per cent of the mosquitoes will be *A. pseudopunctipennis*, and at Ancon during October, 1908, 27 per cent were *A. malefactor* and 72 per cent *A. albimanus*. Mr. A. Busck, of the Bureau of Entomology, United States Department of Agriculture,

who collected and made observations on Zone mosquitoes during 1907, gave it as his opinion that *A. pseudopunctipennis* was the commonest anopheline during the period of his stay.

The necessities of the canal operations in excavating and filling change the topography of districts and localities so as sometimes to convert salt marshes into fresh-water ponds, or to make tracts of land containing few anophelines into a vast swamp in which they luxuriate. On the other hand, swamps and breeding places may be drained or filled in the work of excavation. These factors, among others, influence the number and variety of species in a locality.

The commoner anophelines of the Canal Zone may be divided into three groups:

(A) The white hind-footed group comprising: *A. argyritarsis*, *A. albimanus*, *A. tarsimaculata*.

(B) The leg uniformly-colored group comprising: *A. pseudopunctipennis*, *A. franciscanus*.

(C) The spotted-leg group, comprising: *A. malefactor*, *A. apici-macula*.

These groups present well marked differences in the markings of adults, in the breeding habits and anatomical characters of the larvæ, and, as will be shown, they possess varying susceptibilities to malaria.

The following are descriptions of the species of anophelines of the Canal Zone:

Anopheles argyritarsis.—Thorax with mesonotum bluish-gray, with three more or less longitudinal lines and with pale scales over the mesonotum, and sometimes traces of two dark lateral spots. The abdomen dark, dusky-brown, with a few creamy scales. Legs covered with dark scales, with some of the tarsi apically white banded; last three joints of hind legs pure white, and also apex of first; costa dark, with two distinct and several smaller pale spots.

♀ Head black, with white upright spatulate scales in front, black behind and at the sides, a tuft of white hairs projecting forward between the eyes. Eyes black; antennæ dark, with pale silky pubescence and brown hair; basal joint dark, a few patches of white scales on the first few basal joints; palpi covered with long black scales, especially along toward the base; apex pure white, and there are also two narrow white rings on the apical ends of the joints; ventrally, the penultimate joint has a number of yellowish-white scales, which sometimes seem to form almost a ring; proboscis clothed with short dark scales.

Thorax with a bluish-gray sheen, with three more or less distinct longitudinal lines, the middle one most distinct, and of a purplish hue, with pale scales scattered over the mesonotum; scutellum dark toward the middle; mesonotum deep brown; pleuræ dark, with here and there frosty tomentum (there are traces of two dark lateral spots on the mesonotum, which are clearly seen in the St. Lucia specimen).

Abdomen dusky purplish-brown, clothed with creamy yellow scales, especially in the middle region of the segments; the segments have lateral tufts of gray scales on the posterior borders, projecting from the sides; hairs long, deep bright brown; viewed with a pocket lens the abdomen is almost black in ground color; in other specimens dull yellowish reflections may be seen.

Legs yellowish, covered with dark brown scales; first two tarsi of the forelegs apically white, last two joints dark brown, four mediotarsi also with small pale apical bands; mid metatarsi and first two tarsal joints with minute apical yellow bands, last two indistinctly banded; in the hind legs the last three joints are pure snow white, and also the apex of the first; ungues very dark.

Wings with the costa dark, with four distinct and several smaller white patches; there are also numerous patches of dark scales, which vary to some extent, over the wing areas; in the ♀, from which this description is taken, the fourth long vein is covered with pale dusky scales, whilst in a ♀ from St. Lucia it is creamy white; halteres with pale stem and fuscous knob. Length, 4 to 5 mm.

♂ Palpi dark brown, with scattered white scales, especially on the last swollen joints; hair-tuft pale; there is a pale ring at apex of the apical and base of the penultimate joint; antennæ brown, with brown plumes; proboscis brown and narrow. The white scales on the head extend nearly over the neck; scales on the thorax white; the larger ungues of the four feet biserrated. Length, 4 to 5 mm.

During the period in which these experiments were being conducted I received very few specimens of this species, the sources being Miraflores, Ancon, Culebra, Paraiso, and Corozal. Two specimens of *A. argyritarsis* bit a patient having one crescent to 200 leucocytes and neither became infected. The patient was possibly an unfavorable case and the experiment was not controlled by biting susceptible *A. albimanus* at the same time. On December 2 from some anophelines collected in labor cars at Corozal, one specimen of *A. argyritarsis* was found containing a malarial zygote, 29 μ . in diameter, with fine discrete pigment.

Anopheles tarsimaculata.—This mosquito resembles *A. albimanus* very closely, except for the different arrangement of the white bands on the palpi shown in the drawings. This mosquito was found to transmit malaria.

Anopheles albimanus.—This form resembles the type in all respects except that the last tarsal joint in the hind legs has a very distinct and persistent deep black basal band. The thorax is rather browner in some specimens, and there are only two white bands to the ♀ palpi. The forelegs have dark scaled femora, pale beneath, with a small white knee spot, the tibiæ dusky scaled and also the metatarsus above, pale below, apex white; the first two tarsi have yellow apical bands, the third dark, and the last clay colored; mid legs with a large white spot near the apex of the femora; mid tarsi not definitely banded, but with a faint pale band sometimes at the apex of the metatarsus; the hind legs are dark brown, with the second, third, and apex of the first tarsal joints pure white, the last joint white, with a distinct black basal band; ungues as in the type. Wings much as in the type, but the pale scales are more yellow in color. Length, ♂ 3.5 to 4.5 mm.; ♀ 4 to 4.5 mm.

This was the commonest species of anopheline received as adults or larvæ during the period embraced by this work, and was found to transmit both estivo-autumnal and tertian malaria.

Anopheles gorgasi.—Palpi as long as the proboscis, mostly black scaled, the terminal and penultimate joints light scaled except at the bases and apices; meso-thorax gray with fine brown scales, a black spot in front of the scutellum, a pair of sublateral black spots medially; wings

with the veins scaled in black and white, two very large black patches on the costa and a smaller one toward the base and a smaller one at the apex as in *A. albimanus* Weid. The rest of the wing is too much denuded to describe. Abdomen with groups of outstanding scales laterally at the apices of the segments, the dorsum closed with yellow scales on a dark brown, the lateral tufts black. Legs mostly black scaled, hind legs with the apical half of the second, the third, and the base of the fourth joints white scaled, the remainder of the fourth and basal half of the fifth segments black, the third joint with a large black patch on the underside, which reaches from near the base to beyond the middle. Length, 3.5 mm.

A single adult female of this species was collected by Mr. A. H. Jennings.

Anopheles pseudopunctipennis.—Wings much as in *A. punctipennis* Say, but the fringe with yellow spots. Legs, long, unbanded, brown, pale at the base. Fore ungues of ♂ unequal, mid and hind equal and simple.

♀ Antennæ brown, basal joint testaceous, base of the second joint pale, and also a small pale band at the base of all the following joints: proboscis dark brown, labella yellowish; palpi dark brown, densely scaled at base, apex yellow, and also two narrow yellow bands below, slightly hairy, hairs black, except at the apex, where they are yellow; clypeus dark brown. Thorax yellowish-brown (denuded), with a dark patch on each side of the mesonotum behind; metanotum deep brown; pleuræ yellowish brown, with darker brown patches. Abdomen brown, the segment paler at the base; hairy. Legs deep brown; coxæ, trochanters and base of femora pallid; knee spot pale; ungues equal and simple. Halteres with pale stem and fuscous knob.

Wings with two yellowish white spots on the upper costal border, rest of the edge black, rather densely scaled; first submarginal longer and narrower than the second posterior cell, its stem nearly as long as the cell; mid cross vein a little nearer the base of the wing than the supernumerary cross vein; posterior cross vein still nearer the base of the wing; scales of the wings disposed as follows: First long vein with three distinct large white spots, one at the base, one underneath a large costal spot, and one between; second long vein with a dark patch near its base, all the lower branch of the fork cell dark, and most of the upper; third long vein mostly yellowish-white, with two black patches, one toward the base and the other toward the tip; fourth long vein mostly pale, with two small black patches, branches of the fork cell all dark scaled; fifth long vein with a black spot near the base, rest mostly yellow, upper branch of the fork mostly dark, a small yellow spot at the apex and another toward its base, lower branch mostly yellowish, with a black apical spot; sixth vein with the basal half creamy, the apical half dark, except a small yellow patch where it joins the wing border; fringe brown, with a yellow spot at the junction of each vein. Length, 5 mm.

♂ Last two joints of the palpi swollen and clavate, pale, basal joints dark brown, densely scaled with deep brown scales, with a narrow pale band not quite as long as the thin proboscis, which is brown, with yellow labellæ; antennæ gray, with narrow brown bands and flaxen brown hairs, the apical joint about half the length of the penultimate joint; basal lobe of the genitalia simple, claspers long and thin; fore ungues unequal, the larger one uniserrated, the smaller

minute and simple; mid and hind unguis small, equal, and simple. Wings much as in the ♀, but the fork cells shorter. Length, 5 mm., with proboscis 7.5 mm. Habitat: Grenada. (Doctor Hatyon, per Doctor Daniel.) Time of capture: February.

Observations: Very like *A. punctipennis* Say, but can at once be told by the wing fringe being spotted at the apex of each nerve and by the marking of the sixth long vein. The description is drawn up from two specimens in balsam, so that the scale structure is not evident. It is so very distinct, however, that it can easily be identified by the characters given below. I succeeded in infecting four specimens of this species.

Mr. August Busck found this species to be the commonest and most widely distributed one in the zone during the season in which his collections were made, April–July, 1907.

Anopheles franciscanus.—Male: Head dark brown, with short, dark, erect scales toward nate, emarginate and slightly forked, vertex and anterior part of occiput, with short, light brown scales not forked, a tuft of light brown hairs projecting forward between the eyes, a row of similar hairs projecting forward encircling the eyes posteriorly; eyes deep purplish-brown; antennæ about two-thirds length of palpi, yellowish-brown hairs, basal joint dark brown; palpi equaling proboscis in length with emarginate scales from base to tip on under and outer surfaces, those upon outer surface dark, upon under surface light, long light hairs covering distal third, becoming short and stout at the apex; an area at base of three distal segments, giving a slightly banded appearance; two distal joints spatulate, proboscis scaled except labella, labella covered with medium stout setæ, a few light hairs at apex.

Thorax: Prothorax lobes dark; metothorax dark brown at the sides, with scattered light hairs, a broad light brown patch in the middle; within this light area a median line and obscure lateral lines; scutellum light with single horizontal row of hairs; metanotum dark without hair; halteres dark, covered with thick pubescence and emarginate scales; stalks light without scales.

Abdomen: Basal area of each segment light, covered sparingly with long, light hairs; two stiff hairs on posterior margin of distal segment, stout hairs on margin of genital lobes.

Legs: Coxæ and trochanter light; trochanters, femora, tibiæ, and tarsi covered with short, dark, emarginate scales and setæ; unguis of front legs very unequal, the larger one with a large median tooth and a smaller basal lobe; middle unguis covered, with blunt basal lobes; posterior unguis equal, simple; posterior metatarsus slightly longer than tibiæ.

Wings with dark costa, with two distinct, nearly equal, yellow spots—one at distal end of subcostal vein, one at and involving distal end of first long vein; fringe dark, with a yellow spot at the end of each vein except at the end of the sixth; the first spot carried on to the first long vein, the apical spot carried past over long vein on to the upper branch of the second long vein; the second long vein dark except for a few basal light scales; third long vein yellow in the middle, dark at the base and apex; light area at base of third long vein, carried over the fourth on to the upper branch of the fifth, with a few light scales at base; main branch of fifth long vein light, except at base and apex; distal half of sixth long vein dark, except at apex, basal half light; subcostal

with a light spot carried to the first long vein (in one specimen the light spot on subcostal missing); third long vein prolonged slightly into the basal cell; first submarginal longer and slightly narrower than second posterior cell, stem twice the length of the cell; stem of second posterior cell prolonged to base of wing; supernumerary cross vein adjacent to or but very shortly removed from mid cross vein, and equal to it in length when removed nearer to apex of wing; posterior cross vein a little longer than mid cross vein and varying in distance from it from one half to almost twice its own length; third long vein prolonged slightly into the basal cell, darkest scales on costal, subcostal, and first long veins.

Palpi of the female equaling proboscis in length, light area at base of three distal segments, giving a banded appearance, clothed with scales, short hairs and setæ, as in male, distal joints not spatulate; legs with the ungues equal; otherwise with the male.

No specimens of this species were infected, but as they are so close to *A. pseudopunctipennis* it might have been possible to infect a few if a larger number had been used.

Anopheles malefactor.—♀ Palpi long, clothed with brown scales and black outstanding ones, which are grouped more or less in tufts, heaviest on the basal portion, a slight sprinkling of lighter scales among the brown ones, particularly at the bases of the dark tufts; occiput black scaled, the eyes margined with white above and where they join it a tuft of white hairs; mesonotum gray with reddish and bluish tinge and small dark freckles tending to form longitudinal rows, sparsely distributed narrow yellowish scales, a spot at the base extending over the middle of the scutellum and two small sublateral black spots medially, all three of these show a lighter margin; abdomen slender, gray, with lateral tufts of outstanding black scales at the apices of the segments; legs with the femora and tibiæ black freckled with white, on the hind tibiæ yellow scales predominate; tarsi black, ringed with yellowish-white; on the hind legs the first tarsal joint is dark at the base, light at the apex, and has six white rings of different lengths, second joint narrowly white at base, broadly so at apex, with a moderately broad white ring near the middle and another narrower one between it and the base, third and fourth joints white ringed at base and apex with a broad central white ring, apical segment entirely whitish scaled; wing spotted, black and white, a large black patch margined with white on the costa near the middle, more basally a smaller costal patch and toward the apex another large one, all margined with white, scaling of the veins in patches of black and white scales, the third vein with a small black spot at the base, the sixth vein with many black dots and dashes. Length, 4.5 mm.

♂ Palpi with the apical portion clubbed, clothed with yellow scales with golden luster, a narrow dark ring at the middle of the club, the shaft ringed with dull ochereous at the apex and at the constriction and broadly marked with the same color on the apical portion; antennæ pale brown and ferruginous with silky luster. Length, 4.5 mm.

This large and very beautiful anopheline was received in good numbers from Miraflores and Ancon. Its name, however, appears to be a misnomer, for it could not be infected with malaria under the most favorable conditions.

Anopheles apicimacula.—As in *A. strigimacula* D. & K., but with a distinct black costoapical spot on wing.

[*Anopheles strigimacula*.—Proboscis black; palpi as long as the proboscis, black, a few whitish scales at the bases of the last two and middle of the long joint. Occiput black, clothed with erect black scales, a group of white ones in the center of the vertex, a tuft of pale hairs at the vertex.

Mesonotum narrow, elongate, grayish, pruinose, a black spot below the lateral angle and one on the antescutellar space; vestiture of fine pale hairs arising from small black tubercles. Scutellum collar like, grayish, with a black spot in the middle, clothed with pale bristles. Pleura and coxæ blackish with fine hairs, the coxæ with patches of white scales.

Abdomen with the tip truncate, brownish black, clothed with numerous fine pale hairs; a row of lateral segmental posterior tufts of black spatulate outstanding scales; beneath with tufts of black scales and with scattered white ones.

Wings hyaline, the petiole of the second marginal cell as long as its cell; basal cross vein distant about its own length from the anterior cross vein; scales of the veins ovate, white on the costa and first vein, pale yellow on the others, with black scales and spots as follows:

Three costal spots, the first small involving two veins, the others large, involving three veins, the membrane beneath infuscated; no apical spot; costa and first vein with two or three little black spots between each of the large ones, the outer spot involving the base of the fork of the second vein; each fork with two little spots beyond; third vein with two spots at the base and two at the tip; fourth vein with a spot at the base, a large one involving the base of the fork, three on the upper branch and two on the lower; fifth vein with some black scales at the base, five spots on the upper fork, two on the lower; sixth vein with some irregular black scales toward the base, a spot in the middle and one at the tip.

Legs long and slender, black, speckled with white. Femora with about 8 spots; tibiæ with about 14, being about as many black scales as white ones; hind tarsi with 10 spots on the first joint, second, third, and fourth joints white at the base and tip, with a ring beyond the middle; fifth joint all white. Front tarsi with narrow white rings at bases and apices of the joints, the last entirely pale; mid tarsi not distinctly ringed. Claw simple. Length about 5 mm.; of the wing, 4 mm.]

Anopheles punctimacula.—As in *A. strigimacula* D. & K., but the last vein with a row of black dots.

Only one specimen of this species is known. It was taken at Colon (W. M. Black, collector).

Anopheles cruzii.—This species was examined only in the larval state, kindness of Mr. A. H. Jennings. No adult specimens were obtained and none ever received from or taken from quarters. This is significant on account of the peculiar tree-living habits of this species and the probably groundless fear that it might be a malarial carrier.

Anopheles eiseni.—Near *maculipennis*, but with a patch of whitish scales on the first vein before its middle and another at its apex, also the apical fourth on the hind tibiæ is yellowish-white. Black, the stem of halteres whitish, coxæ and avitta on lower part of pleura, yellow, femora yellowish-brown, apical fourth of hind tibiæ yellowish-

white; antennæ of male whitish, the first joint, last two and fascia on each of the others, brown; scales of palpi black, those of apex and two bands in the female, three in the male, white; scales of occiput black, those in the middle of upper part white; mesonotum grayish pruinose, marked toward each side with a velvet black vitta; scales of abdomen black, the hairs yellowish, scales of femora and tibiæ mixed black and whitish, those on the apical whitish portion of hind tibiæ white, those on the tarsi black; tarsal claws on female simple; wings hyaline, the veins and scales brown, a dense patch of black ones at base of second vein, a larger one on the cross veins and a smaller one at bases of first submarginal and of second posterior cell, a small patch of yellowish-white scales on first vein before its middle and another at its apex, the latter spot encroaching upon the costal vein. Length, 3.5 mm.

It would have been of considerable interest to determine the susceptibility of *A. eiseni* and *A. cruzii* to malaria on account of their peculiar tree-living habits, but it was almost impossible to obtain larvæ of these species; and among hundreds of anophelines caught within quarters and barracks which were examined and dissected no specimens of this species were ever seen. It is extremely unlikely that they play any part whatever in the transmission of malarial fever in the Canal Zone at this time. The larvæ of these species proved extremely interesting in comparison with the commoner species, such as *A. albimanus*, *A. pseudopunctipennis*, and *A. malefactor*, the anatomical characters of the former indicating definitely a very marked alteration in habits.

COLLECTION OF LARVÆ.

In order that a large number of adults could be kept on hand from day to day, it was arranged that sanitary inspectors at the various districts along the Panama Railroad should send bottles containing larvæ and pupæ to the laboratory daily. Special collections of larvæ were also made and excursions to breeding places made from time to time. Upon receiving them at the laboratory, larvæ were transferred to a glass moist jar partly filled with fresh water. Predaceous larvæ, such as dragon fly larvæ, were removed or killed and the anopheles larvæ transferred to feeding tanks containing algæ and organic débris. These glass breeding tanks were placed on a table in front of a window having an eastern exposure, so that they got direct sunlight for a few hours in the morning. The water in the breeding tanks was kept fresh and free from fouling by passing a jet of air through it with a Pacquelin cautery bulb having a heavy glass perforated tip. This proved to be a very important addition to the technique of breeding out larvæ. For shade and shelter a few *Lemna* plants were placed in the tank.

Several writers, collectors, and malarial investigators have mentioned the difficulties attendant upon the breeding out of anopheles mosquitoes from ova or very young larvæ. Others have not mentioned the difficulties encountered or have not described the means used to obviate them. Banks, in the Philippine Journal of Science, volume 2, No. 6, December, 1907, states:

In laboratory breeding experiments the plants in the water begin to die within three to five days, while the larvæ appeared to feed in a desultory manner. The time for

their natural transformation to the pupæ comes and goes and they still remain as larvæ. The foulness of the water, due to organic decomposition, appears not to affect them, but, on the other hand, the lack of proper food seems to cause them to remain in an indefinite larval state until after three weeks or more they gradually begin to die.

A very simple and satisfactory method for keeping the breeding tanks fresh and clean and free from decomposition was devised. The principle involved is that of aeration—preventing the development of an aërobic condition in the water of the breeding tank by passing a fine jet of air through the water of the tank once or twice a day. It is well known, of course, that if natural water from streams, pools, or ditches be placed in a glass tank, exposed to diffuse sunlight, and undisturbed, after a few days the chlorophyll-bearing plants begin to disappear, a pellicle forms on the surface of the water, which contains bacteria, spirilla, flagellated protozoa, amœbæ, etc. Beneath this surface film decomposition goes on rapidly, chlorophyll-bearing forms are destroyed or become encysted, and the various insect larvæ and water bugs die. The bacteria in the deeper portions of the water are largely anaërobes and are associated with the putrefactive decomposition of the vegetable and animal matter in the tank. Banks describes exactly the effect on mosquito larvæ of this putrefying vegetable matter. The odor arising from the tanks suggested the necessity of aeration to prevent the growth of the anaërobic bacteria. Acting on this suggestion, an air jet was devised by attaching a thick glass rod, having a fine capillary central canal, to the double bulb of the Pacquelin cautery apparatus and the breeding-out jars aerated for a minute or two morning and evening. The results were highly satisfactory, for the tanks were by this means kept clean and wholesome. The algæ remained green and vigorous, the larvæ active and developed rapidly into pupæ, the water remained quite clear; in fact, with its floor of sand and clay and a few sprigs of green aquatic plants, such as *Lemna*, looked as tempting to drink as spring water. The temperature of the water in the tanks ranged between 72° and 84°.

BREEDING OUT—METHODS OF FEEDING.

Pupæ were culled in the morning and evening and placed in breeding-out tubes half filled with water and plugged with cotton. Each morning the newly emerged mosquitoes were transferred to biting jars. These biting jars were modified from those described by Stephens and Christophers. After trying pickle jars and malted milk jars with much impatience, jars made of lantern chimneys were used. These were covered on both ends with crinoline gauze, fastened with a string or a strong rubber band. Inside the jar was placed a circular ring platform of stiff paper, which many of the mosquitoes used as a resting place. About 20 mosquitoes would be placed in a jar over a small, slender dish containing water on a Petrie dish cover with a raisin or a piece of date for food. The jar would then be placed on a shelf in a dimly lighted place, protected from ants by kerosene cups. Adult mosquitoes may be kept alive for several days in such jars if they are fed with dates or raisins and a few drops of water, but if fed daily they will not bite and suck blood with alacrity. Anophelines do much better if they have one or two preliminary blood meals before being fed with dates, raisins, or bananas. In several experiments it appeared that in the midgut of

mosquitoes gorged with stale banana and the associated bacteria and yeasts the fermentative acid contents either destroyed the flagella of gametes or in some other way prevented the development of zygotes in the mid gut of susceptible mosquitoes. In one experiment a patient whose blood contained almost as many crescents as leucocytes was bitten by four varieties of mosquitoes, three of the four being susceptible species; yet none became infected. This failure might be attributed to the feeding of bananas.

Until the mosquitoes were used for biting they were fed with dates, raisins, and bananas. Whenever they were to be kept for biting and dissections, it was found that those fed on dates and raisins gave much better dissections, while the mid guts of banana-fed mosquitoes frequently contained many wild yeast and bacilli which caused the death of some of the mosquitoes, apparently as a result of fermentation, and frequently yielded partly softened, tender, and disintegrated intestinal tracts, which were removed intact with difficulty and were occasionally lost. Bananas should never be used as food when dates or raisins can be obtained.

Mosquitoes were kept in these biting jars until a suitable case of malaria presented. The blood of patients selected to be bitten contained gametes or sexual forms of the malarial parasite in their circulating blood in numbers sufficient to infect susceptible anophelines. The method of determining this point will be developed later.

BITING AND INFECTING EXPERIMENTS.

The earlier biting experiments were conducted at about 8 o'clock in the evening. After selecting a patient the jar of mosquitoes was placed on the patient's forearm and covered with a heavy towel to prevent the disconcerting effect of light. Females several days old, that had been fed exclusively on dates or raisins, would generally bite greedily, and would feed on successive or alternate nights if given an opportunity. Most females would bite twenty-four or more hours after emerging, but before that period they would generally make no attempt to do so. When a jar is placed over a patient's forearm, the mosquitoes that are going to bite will almost always do so within a few seconds. If they show no inclination to visit the arm, a few gentle puffs into the opposite end of the jar, through the crinoline gauze will make them change their position and frequently take up one on the patient's forearm. Another method that was used successfully was to have the patient's bed in the dark. If the jar were then gently tapped two or three times and aimed at a light down the ward some distance away, the mosquitoes would always take up a position on the gauze facing the light. The patient's forearm could then be carefully interposed and placed on the gauze, when the mosquitoes would frequently take the hint and feed. Strong lights are powerfully attractive to female anophelines and interfere considerably with successful bitings unless the jars are darkened by means of a thick cloth, the patients taken into a dark room, or the mosquitoes very hungry.

Later, it was more convenient to conduct the bitings about 4 o'clock in the afternoon, and on these occasions it was generally necessary to cover the jars well with a thick towel.

Two classes of patients were used, Spanish laborers and West Indian negroes; the former rarely objected to being bitten, the latter occasionally fretted a little. Both classes of patients were rather obtuse mentally and occasionally would not feel the bite, or would not be able to tell correctly the number of them. One reason for this is to be found in the limits of the number of separate points of pain appreciable within a radius of 3 inches, the biting area of the jar. The Spanish patients' hands occasionally reeked with the odor of cigarette smoke and a few of the West Indians had used citronella to prevent sand flies from biting, but in neither instance did this interfere with the feeding of mosquitoes. The hungry females, if undisturbed, will gorge themselves with blood and not infrequently will expel a drop or two of bloody fluid per anum. This not only occurs at the first feeding but has been observed at subsequent feedings.

After as many of the females as will bite have done so, the jars are taken to the laboratory, one of the gauze covers carefully removed and at the same time replaced by a card, which is slipped out when the jar is placed on a petrie dish. The petrie dish contains a small stender-dish with a few drops of water and a split raisin or two. The jar is then placed on an ant-proof shelf in a dimly lighted place, and under these conditions, with fresh food and water, anophelines may be kept alive for two or three weeks. If one has bitten mosquitoes late in the evening and does not care to transfer or uncover them by artificial light, the gauze may be moistened with a few drops of tap water and the jars placed on the shelf until morning.

At the time of biting the patient, two or three good blood films for staining were taken and differential counts of leucocytes as well as the proportion of gametes to leucocytes made the following morning. By this means, and by an occasional leucocyte count, it was possible to estimate from films the number of gametes ingested by the mosquito in feeding.

ESTIMATION OF GAMETES.

The estimation of the number of gametes ingested by the mosquito gives one an idea as to the grades of infection to be encountered in the mosquito, and this has indirectly a practical bearing which will be discussed later.

Leucocyte counts taken during convalescence in malarial fever generally show a number not far removed from the normal. It was possible, then, to estimate the number of gametes by counting the number met with while enumerating the leucocytes during the differential count. Such a gamete count was made from the blood of every patient bitten.

Mosquitoes were weighed before biting and after biting, and the amount of blood ingested estimated in this way:

	Weight.
Albimanus bred in laboratory, twenty-four hours old, midgut empty.....	0.0008
Albimanus bred in laboratory, moderate blood feeding.....	.0016
Albimanus caught in labor cars, some blood in midgut, half-developed ova...	.0019
Albimanus caught in labor cars, much blood and early development of ova...	.0035
Albimanus caught at barracks, blood in midgut: no development of ova.....	.0018
Albimanus caught at barracks, blood in midgut: no development of ova.....	.0021

The average weight of mosquitoes twenty-four hours old was 0.0008 gramme, while the average weight of mosquitoes from the

same brood that had bitten and taken a moderate amount of blood was 0.0016 gramme. The average weight of blood ingested being then 0.0008 gramme—approximately 0.001 gramme. Assuming the specific gravity of blood in malarial fever with slight anæmia to be 1.050, then: $105:100::0.0008:0.000761$ = the volume of 0.0008 gramme of blood. Now if there were 22 gametes per 100 leucocytes, as in experiment 38, and 6,500 leucocytes per one cumm., as there were, by actual count, there would be $22 \text{ by } 65 \text{ by } 0.761 = 1,088$ gametes ingested. If, under the most favorable circumstances, there are an equal number of male and female gametes, there should have been about 1,632 zygotes in this mosquito's mid gut after three feedings, but as a matter of fact, there were only about 50, showing a loss of about 97 per cent. This loss may be partly explained by an observation on the fate of gametes in *vitro* and in *vivo*, when it was noticed that fully 50 per cent of gametes were phagocyted by polymorphonuclear leucocytes. This last is truly surprising, because this type of leucocyte in the circulating blood of man rarely plays any part in malarial phagocytosis, save in pernicious infections, when it will then engulf parasites and pigment. Usually, it is the large mononuclear and endothelial cells which phagocyte malarial parasites and pigment.

In experiment 41 blood specimens from a patient taken on admission contained 92 crescents per 100 leucocytes. Fresh preparations examined fifteen to twenty minutes after being taken, contained many pigmented extracellular parasites, some with quiescent pigment, others with pigment dancing in a circle around a granular center. There were free flagella in some fields—two in one field. One flagellum was seen attached to several red blood cells and was moving with extreme violence, but without becoming detached. Quite a number of the gametes became phagocyted by polymorphonuclear leucocytes, one of the latter had phagocyted two gametes. Stained specimens showed numerous gametes within the polymorphonuclear leucocytes, one free detached flagellum was seen. Some of the gametes were globular with linear pigment and a large chromatin dot, while others contained granular pigment, surrounding a chromatin ring, staining interruptedly and having an acromatic space on its interior and exterior.

CARE OF MOSQUITOES AFTER BITING.

The mosquitoes may be fed nightly or every other night from patients, or, if it is desired to ascertain the rate of development of zygotes, they are fed on dates after a single biting. The mosquitoes should be kept in the biting jars with fresh food and very little water, just enough to favor oviposition and to keep the air within the jar moist, but not so much that they would be drowned or sprawled.

It is very necessary that the infected mosquitoes be protected from ants, otherwise valuable specimens that die during the night will be removed with nothing but wings and legs to mark the loss.

Mosquitoes may be transferred one at a time from the biting jar and from day to day killed and dissected.

Chloroform and cyanide may be used for killing. Chloroform is more conveniently used, but cyanide yields better preparations when it is desired to preserve most of the mosquito for identification, for the reason that cyanide causes the mosquitoes to spread their wings.

METHOD OF EXAMINING FOR SPOROZOITES AND ZYGOTES.

First it should be said that successful dissections can only be obtained with killed specimens. Mosquitoes that have been dead twelve or more hours, particularly if they had fallen on the water, have become so macerated that the cells of the midgut of salivary glands separate and float away, it being impossible to retain the organ intact. When an examination of the salivary glands is desired the wings and legs of the mosquito are trimmed off with a small sharp entomological knife. The distal half of the thorax, with the abdomen, is removed by a transverse clean cut, the mosquito being laid on a piece of white cardboard. With a small pair of forceps the proboscis is grasped and the specimen laid on a drop of saline solution on a stender-dish cover, under the dissecting microscope. The chitinous covering of the thorax, just behind the nape, is carefully slit, or torn, and the muscle organ beneath loosened slightly; then by pulling out the proboscis with one needle and holding the chitinous thoracic covering with the other, the salivary glands will be drawn out. They should be cut off from the head by a small, very sharp knife, and picked up with the point of a bright needle and placed in saline solution, 10 per cent formalin, or films made for staining.

The sporozoites will be seen either free or in epithelial cells, and in the duct of the salivary glands, appearing as thin, slightly curved, spindle-shaped bodies, placed side by side, frequently as though matted together.

METHOD OF EXAMINING FOR ZYGOTES.

The identified mosquito is laid on a piece of white cardboard, the abdomen cleanly and carefully removed by a transverse cut, just behind the thorax, and placed on a glass slide; or, better, the under surface of a stender dish cover, with a drop of saline solution. Under the dissecting microscope with the reflector properly adjusted, the hind gut, mid gut, malpighian tubules and ovaries are withdrawn by pulling carefully on the last abdominal segment with one needle and holding the first abdominal segment by a corner with another needle. The mid gut is separated from the hind gut and malpighian tubules as well as possible and transferred to a slide on the point of a needle, which should be sharp and well burnished, where it may be examined in saline solution, formalin, or by other methods. When the mosquito has been dead several hours the mid gut can not be withdrawn intact. In this event, it is generally necessary to split the chitinous abdomen open, and search carefully for as much of the mid gut as may have held together. Zygotes can be detected with a low power lens Zeiss, 16 mm. objective and 8 and 12 oculars. If an absolute identification of the mosquito can not be made at the time, all parts excepting the abdomen must be preserved intact, mounted with experiment number attached for final identification.

DESCRIPTION OF THE MALARIAL PARASITE IN THE MOSQUITO.

As stated above, it was found that many gametes were phagocyted in the mosquitoes' stomachs (50 per cent or more) by a polymorphonuclear leucocytes. This materially diminishes the number of fertilized ookinets which reach and enter the wall of the mosquito's

mid gut. Notes from the following experiment throw some light on the changes occurring in crescents *in vitro*. The phagocytosis of crescents in the mid gut was demonstrated separately, but films do not permit as accurate study as those *in vitro*.

EXPERIMENT No. 20.

Blood contains, December 30, 67 crescents per 100 leucocytes. Temperature 97° continuously.

Differential count of leucocytes:

Poly.....	68
Large.....	4
Small.....	a 20
Eosinophiles.....	7
Mast.....	1

In the fresh blood film, when it is drawn, numerous crescents are seen, but within five to ten minutes half the number of crescents have become vesicular and their pigment dancing; several have thrown out flagella, which are still attached. After fifteen to twenty minutes these vesicular gametes have become phagocyted by polymorphonuclear leucocytes; some of them at this period have parted with their flagella because several free flagella were detected in the film. Stained preparations fixed immediately upon drawing the blood show nothing but crescentic forms, but films that have been kept moist from five to ten minutes before staining, show that a very marked change has taken place in the crescents, many of them becoming either globular or irregular and distorted. One film fixed after ten minutes, and stained, showed 9 crescentic forms, 8 globular forms, with discrete pigment, and several chromatin dots. Some of these are certainly microgametes, and 11 irregular forms, as though becoming globular, or as crescents losing their stiff outline, and becoming flexuous.

After fertilization the microgametocyte becomes elongated (wandering vermicule, ookinet). Illustrations of wandering vermicules (after Grassi) are very much like the 11 irregular forms seen in the blood just described. These, then, may represent fertilized gametes.

The fertilized gamete, or ookinet, if it be not phagocyted, has abundant time to wander out of the blood clot and reach the gut wall, for the blood meal of the mosquito is usually not expelled until after twenty-four hours.

The earliest form of the estivo-autumnal zygote was detected in the wall of the gut after the expulsion of the blood meal, or after two and one-half days. Satisfactory dissections and examinations can not be made until the blood meal has been expelled; consequently, after several trials, sixty hours after a feeding, was the earliest period at which a search was made for zygotes.

In experiment No. 204 a specimen of *A. tarsimaculata* was killed sixty-five hours after a single feeding from a patient whose blood contained 10 crescents per 100 leucocytes. Upon examination there were about 50 zygotes in the mid gut. They were slightly oval in outline, with closely clumped quiescent pigment, and very little cytoplasm showing beyond the pigment, the diameter being about

a 67 crescents per 100 leucocytes.

5 mu. The zygotes become larger each day, though they do not always appear to grow at equal pace.

This variation in size may depend on location in the gut wall and its relation to nutrition. On the whole, however, the gradual increase in the size of the zygote is fairly uniform, as the following table shows:

Experiment No.	Age of zygote.	Type of parasite.	Size of zygote in mu.
	<i>Days.</i>		
16	4-4½	Tertian.....	12 x 16.5.
16	4-4½	do.....	17 x 20.
32	5	do.....	19.5.
16	8½-9	do.....	48, 54.
16	8½-9	do.....	48 x 54 x 55.5 x 66.
18	(a)	do.....	(b)
204	c 65	Estivo-autumnal..	5.
9	c 60	do.....	9 x 11.25.
41	2½	do.....	9 x 10.5.
38	3	do.....	7 x 10.
43	3	do.....	7 x 10.
41	3½	do.....	12 x 15.
38	4	do.....	12 x 15.
33	3¾-4¾	do.....	12 x 13.5.
42	4¾	do.....	21.
36	5	do.....	21.
11	6½	do.....	22, 28.
38	7	do.....	45 x 57. ^d
13	7½	do.....	32 x 40.
13	8½	do.....	39.
36	11	do.....	(a)
17	12½	do.....	e 30.

a Sporozoites in salivary glands.

b Sporoblasts formed.

c Hours.

d Contains sporoblasts.

e No sporozoites in salivary glands.

Measurements were made from fresh preparations in 10 per cent formalin-saline under cover slip with ocular micrometer (Zeiss).

The zygotes, as will be seen from the foregoing table, generally assume an ovoidal shape, one diameter being a little longer than the other. As they increase in size they become more vesicular, the periphery assumes a definite rim-like contour, and the pigment becomes more and more scattered, discrete, and ultimately, inconspicuous. During the first sixty hours the pigment is tightly clumped, but as the zygote becomes larger and vesicular, the pigment usually spreads out in lines or belts, sometimes in small clumps, or occasionally scattered and discrete. In experiment No. 38, a specimen of *A. albimanus* contained about 50 estivo-autumnal zygotes, three and one-half days old (12 by 12 mu), in its mid gut; the pigment was in the form of linear rods, and almost every zygote had a zonal arrangement of its pigment. The zone of pigment was made up of rods, end to end, each rod separated by a small space; sometimes the zonal pigment was concentric with the periphery of the zygote, sometimes at right angles to the plane presented to the observer, but apparently always peripheral.

In experiment No. 33, a specimen of *A. albimanus* contained 12 to 15 estivo-autumnal zygotes, three and three-fourths to four and three-fourths days old, 12 by 13.5 mu in size, mostly oval in outline. The pigment was bronze in color, and in clumps, never in lines or belts. In experiment No. 36, a specimen of *A. albimanus* contained estivo-autumnal zygotes five days old, 21 mu in diameter, with the

pigment present in clumps. On counting the number of pigment rods in a zygote, and in crescents, it is evident that conjugation of gametes does not occur, for there is the same amount of pigment in each instance. In experiment No. 42, a specimen of *A. albimanus* contained several estivo-autumnal zygotes of three ages (three bitings from the same patient). Five or six zygotes were between one and one-half and two and one-half days old, and their pigment, of course, tightly clumped. One zygote, 21 μ in diameter, was globular and contained three clumps of pigment, while in several others of the same age the zygotes were ovi form and the rods of pigment were arranged in pairs, scattered irregularly throughout the zygote, each one containing 13 or 14 rods of pigment.

In most of the zygotes, excepting the very young or very old forms, the pigment was arranged in lines or belts, but not uncommonly were seen zygotes of equal age in the same specimen, some with belts of pigment and some having it in clumps.

The larger zygotes, containing sporoblasts, showed very little pigment. This is collected in one clump, usually near the periphery. The pigment is probably not destroyed nor extruded, but is partly obscured by the greater size of the zygote.

Some of the smallest zygotes contained dancing pigment, and in the larger zygote, with zones of linear pigment, the zone could be seen to change its position very slowly, but in these instances the pigment was not dancing.

When the zygotes reach the size of 39 by 54 μ , or 45 by 57 μ (seven to eight and one-half days), a fine reticulum could be seen outlining the sporoblastic chamber, while others were dotted throughout with coarse round granules, the sporoblasts.

The capsule of the zygote ruptures about the eleventh day, the sporozoites making their way to the salivary glands, and leaving the collapsed, wrinkled, disk-shaped envelopes behind in the outer layer of the midgut wall. The mechanism of the passage of the sporozoites into the salivary glands is not known, but these glands are more or less filled with sporozoites after the eleventh day.

The tertian zygote differs from the estivo-autumnal first in the rapidity of its development, it obtaining a slightly greater size in a given time. In the younger zygotes the pigment is arranged in clumps or lines and slowly changes its position. The pigment may be clumped and dancing within vacuolated spaces of the zygote. In experiment No. 16, tertian zygotes in *A. albimanus*, were 12 to 16.5 μ in diameter, four to four and one-half days after biting. The capsule of the zygote was well defined, but the zygote was apparently slightly larger than an estivo-autumnal zygote of the same age, and its cytoplasm was more coarsely granular than that of the estivo-autumnal parasite. The pigment is coarse and in clumps, not lines, and is not motile. In another specimen of *A. albimanus*, in the same experiment, the pigment was arranged in two lines, near the periphery of the zygote. Nine days after the first biting, one *A. albimanus*, with half mature ova, contained six to eight zygotes, full of faintly refractile, equally sized sporoblasts, 3 μ in diameter. These zygotes were 48 μ in diameter. Four or five zygotes were seen projecting a portion of their rotundity from the outer wall of the gut. They were 48 by 54 μ , and 55.5 by 66 μ . No pigment could be detected, and there were faint clusters in groups, having a linear

arrangement, indicating the development of sporozoites from sporoblasts. In this experiment, the zygotes nearly reached maturity in nine days.

In experiment No. 18, tertian sporozoites were found in only one of five acini of the salivary glands in a specimen of *A. albimanus*, eleven and one-half days after the first biting. Four other acini were in plain view, but were entirely devoid of sporozoites. The sporozoites in the infected acinus were distributed in the lumen of the duct and in several of the distended, vesicular epithelial cells, several hundred being present, their long axis being generally parallel with the duct.

In experiment No. 36, estivo-autumnal sporozoites were found after the eleventh day in the red hyaline salivary acini on both sides of the body in large numbers. Very few in the faintly stained, larger vacuolated acini, and none in the acini containing colloid globules; but the duct of the latter contained sporozoites, which were generally matted together.

Table showing data in relation to infected and noninfected mosquitoes:

Experi- ment No.	No. and species of mos- quito. ^a	Type of parasite.	No. of game- tes.	Dissected days after biting.	No. of zygotes present.	Con- trolled by albi- manus.	Date of experi- ment.	No. of patient.	Ova de- veloped.
2	1 M.	Quartan malaria.	5	3	0	No.	Oct. 13	9 18 B	(?)
3	1 A.	Estivo-autumnal.	10	3½	0	Yes.	Oct. 19	47496	No.
3	1 A.	do.	10	3½	5	Yes.	do.	47496	Yes. ^b
3	1 A.	do.	10	4½	0	Yes.	do.	47496	No.
3	1 A.	do.	10	4½	0	Yes.	do.	47496	No.
4	1 P.	do.	±14	5	±5	No.	Oct. 27	48132	No.
4	1 P.	do.	±24	5	31	No.	do.	48132	No.
5	1 M.	do.	3.5	4½	0	No.	Nov. 10	48509	No.
5	1 M.	do.	3.5	4½	0	No.	do.	48509	No.
5	1 M.	do.	3.5	4½	0	No.	do.	48509	No.
5	1 M.	do.	3.5	4½	0	No.	do.	48509	No.
6	1 M.	do.	16	3½	0	Yes.	Nov. 11	48987	Yes.
6	1 M.	do.	16	3½	0	Yes.	do.	48987	No.
6	1 M.	do.	16	3½	0	Yes.	do.	48987	No.
9	1 P.	do.	27	2½	0	No.	Nov. 24	48987	No.
9	1 P.	do.	27	2½	6 or 7	No.	do.	48987	No.
10	1 A.	do.	12	4	(c)	Yes.	Nov. 27	48987	No.
11	1 A.	do.	8	7	0	Yes.	Nov. 30	48987	No.
11	1 A.	do.	8	7	(d)	Yes.	do.	48987	No.
11	1 A.	do.	8	7	168	Yes.	do.	48987	No.
12	1 P.	do.	8	2½	0	Yes.	do.	48987	No.
13	1 P.	do.	20	3½	0	Yes.	Dec. 4	48987	No.
13	1 P.	do.	20	3½	6 or 7	Yes.	do.	48987	No.
13	1 A.	do.	20	7½	3	Yes.	do.	48987	Slightly.
13	1 A.	do.	20	8½	(c)	Yes.	do.	48987	No.
13	1 A.	do.	20	8½	(c)	Yes.	do.	48987	No.
16	1 A.	Tertian.	+3	4	6 or 8	Yes.	Dec. 21	51147	No.
16	1 A.	do.	+3	4½	±20	Yes.	do.	51147	No.
16	1 A.	do.	+3	8½	6 or 8	Yes.	do.	51147	Yes.
16	1 A.	do.	+3	8½	4 or 5	Yes.	do.	51147	(?)
18	1 A.	do.	3	11½	1	Yes.	Dec. 27	51431	Yes.
32	1 A.	do.	±17	4½	2	Yes.	Jan. 25	53343	Yes.
32	1 A.	do.	±17	4½	0	Yes.	do.	53343	Yes.
32	1 Ag.	do.	±17	4½	0	Yes.	do.	53343	Yes.
32	1 P.	do.	±17	4½	0	Yes.	do.	53343	Partly.
32	1 P.	do.	±17	4½	0	Yes.	do.	53343	Do.
32	1 P.	do.	±17	4½	0	Yes.	do.	53343	Do.
32	1 P.	do.	±17	4½	0	Yes.	do.	53343	Yes.
33	1 A.	Estivo-autumnal.	11	4½	12-15	Yes.	Jan. 26	H. G.	Yes.
33	1 A.	do.	11	4½	12-15	Yes.	do.	H. G.	Yes.
34	1 P.	do.	4	4½	0	No.	Jan. 27	H. G.	No.
35	1 Ag.	do.	8	2½	0	No.	Jan. 28	53499	No.
36	1 A.	do.	27	5	(d)	Yes.	Jan. 30	53499	Yes.
36	1 P.	do.	27	5	0	Yes.	do.	53499	No.
36	1 P.	do.	27	6	0	Yes.	do.	53499	Yes.

^a A=albimanus; M=malefactor; T=tarsimaculata; P=pseudopunctipennis; Ag=argyritarsis.

^b Caught in barracks.

^c Numerous.

^d Present.

Experi- ment No.	No. and species of mos- quito.	Type of parasite.	No. of game- tes.	Dissected days after biting.	No. of zygotes present.	Con- trolled by albi- manus.	Date of experi- ment.	No. of patient.	Ova de- veloped.
36	1 P.....	Estivo-autumnal.	27	6	0	Yes...	Jan. 30..	53499	Yes.
36	1 A.....	do	27	11	(a)	Yes...	do	53499	Yes.
36	1 A.....	do	27	13½	(a)	Yes...	do	53499	
37	1 P.....	do	29	3	0	Yes...	Feb. 1	53742	No.
37	1 P.....	do	29	3	0	Yes...	do	53742	No.
37	1 P.....	do	29	3½	0	Yes...	do	53742	Yes.
37	1 P.....	do	29	3½	0	Yes...	do	53742	Slightly.
37	1 P.....	do	29	3½	0	Yes...	do	53742	Do.
38	1 A.....	do	29	2¾	(a)	Yes...	do	53742	No.
38	1 A.....	do	29	3½	(b)	Yes...	do	53742	Slightly.
38	1 A.....	do	29	6¾	(c)	Yes...	do	53742	Yes.
38	1 P.....	do	29	11½	0	Yes...	do	53742	No.
38	1 A.....	do	29	11½	(b)	Yes...	do	53742	Slightly.
41	1 A.....	do	92	3½	(c)	Yes...	Feb. 5	53937	Yes.
41	1 A.....	do	92	8½	(?)	Yes...	do	53937	(?)
41	1 A.....	do	92	10½		Yes...	do	53937	
44	1 P.....	do	3.5	1½	0	No...	Feb. 14	53937	No.
44	1 M.....	do	3.5	2¾	0	No...	do	53937	No.
44	1 M.....	do	3.5	4½	0	No...	do	53937	Yes.
44	1 M.....	do	3.5	4½	0	No...	do	53937	No.
44	1 P.....	do	3.5	4½	0	No...	do	53937	Slightly.
46	1 A.....	do	2	1½	0	Yes...	Feb. 17	53937	No.
47	1 P.....	do	.5	3½	0	Yes...	Feb. 19	53937	No.
47	1 P.....	do	.5	3½	0	Yes...	do	53937	No.
47	1 A.....	do	.5	3½	0	Yes...	do	53937	Slightly.
47	1 A.....	do	.5	3½	(?)	Yes...	do	53937	Yes.
47	1 A.....	do	.5	3½	No.	Yes...	do	53937	Yes.
47	1 A.....	do	.5	3¾	3	Yes...	do	53937	No.
47	1 A.....	do	.5	3¾	4	Yes...	do	53937	No.
47	1 P.....	do	.5	3¾	0	Yes...	do	53937	No.
47	1 M.....	do	.5	3¾	0	Yes...	do	53937	No.
47	1 A.....	do	.5	3¾	0	Yes...	do	53937	Slightly.
47	1 M.....	do	.5	3¾	0	Yes...	do	53937	No.
47	1 P.....	do	.5	3¾	0	Yes...	do	53937	No.
47	1 P.....	do	.5	3¾	0	Yes...	do	53937	No.
47	1 P.....	do	.5	3¾	0	Yes...	do	53937	No.
42	1 A.....	do	10	1½	0	Yes...	Feb. 6	53742	No.
42	1 A.....	do	10	1½	0	Yes...	do	53742	No.
42	1 P.....	do	10	1½	0	Yes...	do	53742	No.
42	1 A.....	do	10	3¾	(d)	Yes...	do	53742	Yes.
42	1 M.....	do	10	3¾	0	Yes...	do	53742	Yes.
42	1 A.....	do	10	4½	(a)	Yes...	do	53742	Yes.
42	1 A.....	do	10	4½	(a)	Yes...	do	53742	Yes.
42	1 A.....	do	10	5¾	(b)	Yes...	do	53742	Yes.
42	1 A.....	do	10	5¾	(b)	Yes...	do	53742	Yes.
42	1 A.....	do	10	5¾	3 or 4	Yes...	do	53742	Yes.
42	1 A.....	do	10	5¾	3 or 4	Yes...	do	53742	No.
43	1 A.....	do	5	2¾	5	Yes...	Feb. 8	53742	No.
43	1 M.....	do	5	2¾	0	Yes...	do	53742	No.
47	1 P.....	do	.5	5½	0	Yes...	Feb. 19	53937	No.
47	1 M.....	do	.5	5½	0	Yes...	do	53937	No.
47	1 Ag.....	do	.5	5½	0	Yes...	do	53937	No.
47	1 Ag.....	do	.5	5½	0	Yes...	do	53937	No.
47	1 M.....	do	.5	5½	0	Yes...	do	53937	No.
202	1 P.....	Quartan malaria.	0(?)	4	0		Aug. 24	Bed 90	Yes.
202	1 T.....	do	0(?)	4	0		do	Bed 90	Yes.
204	1 T.....	Estivo-autumnal.	±5	3	±40	Yes...	Aug. 28	63472	Yes.
204	1 A.....	do	±5	4	(b)	Yes...	do	63472	No.
204	1 A.....	do	±5	9	(b)	Yes...	do	63472	Yes.
209	1 T.....	do	±5	2	20	Yes...	Oct. 5	65343	Yes.
209	1 T.....	do	±5	2	22	Yes...	do	65343	Yes.

a Present.*b* Many.*c* Numerous.*d* Several.

NOTES AND CONCLUSIONS FROM THE FOREGOING TABLE.

Species.	Number.	Infected.	Per cent infected.
<i>A. malefactor</i>	17	0	0.0
<i>A. pseudopunctipennis</i>	31	4	12.9
<i>A. albimanus</i>	48+2	34+2	70.2
<i>A. argyritarsis</i> <i>a</i>	4	0	0.0
<i>A. tarsimaculata</i>	5	3	60.0

a A naturally infected specimen of this species has been found in barracks.

Out of several hundred mosquitoes used in the biting experiments, 107 gave satisfactory dissections, or paraffine sections, and it was determined that 70.2 per cent of *A. albimanus* became infected; 60 per cent of *A. tarsimaculata* became infected; and 12.9 per cent of *A. pseudopunctipennis* became infected; while none of *A. malefactor* became infected, although several were placed in jars with *A. albimanus* and bit at the same time persons from whom the specimens of *A. albimanus* became infected.

It is concluded from this series of experiments that *A. albimanus*, the common white hind-footed mosquito—an extremely hardy, rapidly developing, adaptable mosquito, is the transmitter of estivo-autumnal and of tertian malarial fever in the Canal Zone at this time. Specimens of this species infected with tertian parasites became infective between nine and eleven and one-half days after the first feeding. When infected by estivo-autumnal parasites, sporozoites appeared in the salivary glands as early as the eleventh day in some mosquitoes, and later than twelve and one-half days in others.

A. tarsimaculata appears to be as susceptible to the estivo-autumnal parasite as *A. albimanus*, and no doubt if a favorable opportunity had presented it would have been found that *A. tarsimaculata* would have been equally susceptible to tertian malaria.

A. pseudopunctipennis is only slightly concerned in the transmission of malarial fever, if at all, not only from the fact that only 4 out of 31 mosquitoes, under the most favorable artificial conditions, became infected, but from the additional fact that relatively few specimens are taken in quarters at this time.

A. malefactor is not concerned in the transmission of malarial fever in the Canal Zone at this time.

Out of 41 mosquitoes containing malarial zygotes, 17 showed no development of ovaries or ova. A few of these, however, might have oviposited before examination.

Out of 60 negative bitings, i. e., where mosquitoes of any of the species bit and failed to become infected, 20 showed development of ova; and to take the known susceptible species, *A. albimanus* and *A. tarsimaculata*, out of 13 *A. albimanus* and *A. tarsimaculata*, which failed to become infected, 6 showed development of ova.

It would, therefore, appear that fecundation and development of ova are not necessary for the development of zygotes.

Under the most apparently favorable circumstances one or two out of the lot of susceptible anophelines will fail to become infected and these, no doubt, possess an active immunity toward the malarial parasite.

LIMITS OF INFECTIOUSNESS OF MAN.

During the progress of the experiments it was noticed that patients were discharged after their temperature had become normal and when their peripheral blood occasionally contained more than a sufficient number of gametes to infect susceptible mosquitoes. In order that a recommendation might be made for the continued treatment of these persons, it was necessary to determine, if possible, the limits of infectiousness of such individuals. Several experiments were carried out, in which *A. albimanus* bit patients whose gametes per leucocytes had been determined. These mosquitoes were given

but one blood feeding and fed subsequently on dates and raisins and then dissected. The limits were determined as being near 1 gamete per every 500 leucocytes, or 12 gametes per 1 c. m. m., but it must be understood that several factors are concerned in infections, such as number and phagocytic power of leucocytes; immunity of mosquitoes, racial and individual; probable reaction of gut contents, as acid, bacterial products or those from yeasts, may be inimical to the gametes; the proportion of ♀ to ♂ gametes plays some part. This, however, was not worked out at this time, besides the number of gametes ingested. In experiment 20 and 28 patients' blood was rich in crescents which flagellated *in vitro*, and in the mosquito's stomach, yet mosquitoes never could be infected from the patients.

Persons with more than 12 gametes per 1 cm. must be regarded as gamete carriers, and, of course, should not be discharged from hospital nor should treatment be discontinued until gametes have been reduced well below the limits of infectiousness. This destruction and prevention of the development of the sexual forms of the parasite in man is a matter generally overlooked, but is of the greatest importance in delimiting malaria, and it may be accomplished by appropriate quinine treatment of all gamete carriers; by quinine treatment to destroy latent malaria and by periodical blood examination of laborers and others in quarters where there is a high malarial rate. For the detection of gamete carriers and latent malaria, in order to carry out appropriate treatment, 30 grains of quinine sulphate in solution daily is an efficient dosage for the purpose required.

Experiment No. 47, February 19, 1909, 8 p. m., jar containing *A. albimanus*, *A. pseudopunctipennis* and *A. malefactors* were applied to No. 53937, who was receiving 30 grains of quinine daily when his blood contained 1 crescent to 200 leucocytes = approximately $30 \pm$ gametes per 1 c. m. m. The mosquitoes were given but one blood feeding and subsequently fed on raisins and dates.

Upon dissection on February 23-25, 9 *A. pseudopunctipennis* were not infected, 4 *A. malefactors* were not infected, 2 *A. argyritarsis* were not infected, 3 *A. albimanus* were not infected; while 1 *A. albimanus* contained 3 zygotes and 1 *A. albimanus* contained 4 zygotes.

Experiment No. 31, January 25, 1909, jar containing *A. pseudopunctipennis*, *A. argyritarsis* and *A. albimanus* were fed from 53343, tertian malarial fever, whose blood contained 4 gametes per 100 leucocytes.

Upon dissection January 30, 3 *A. pseudopunctipennis* were not infected, 1 *A. argyritarsis* was not infected, 1 *A. albimanus* was not infected; while 1 *A. albimanus* contained 2 zygotes.

NOTES ON THE BIONOMICS OF SOME OF THE ANOPHELINES STUDIED.

The period of incubation of the ova of *A. albimanus*, *A. pseudopunctipennis*, and *A. malefactor* was estimated as about thirty-six hours under the laboratory conditions, the temperature of the air and water ranging between 78° and 82° daily, an eastern window exposure with direct sunlight for three or four hours in the morning, and diffused sunlight the remainder of the day. The eggs are laid in the geometrical patterns usually seen, and at first are creamy-white color, becoming in a few hours quite black, with white lateral air chambers.

The larval period varies with the species, food, efficient temperature, sunlight, and environment; for example, ova of *A. malefactor* and *A. albimanus*, of about the same age, were exposed in the same breeding tank to an identical environment, food, water, air, and sunlight; but when *A. albimanus* had pupated the larvæ of *A. malefactor* were only half grown. In one experiment, *A. albimanus* larvæ pupated within twelve days, while *A. malefactor* required sixteen to twenty days. Subsequent examinations of water from *A. malefactor* pools, and from the intestinal tracts of malefactor larvæ, indicate that the latter prefer shady pools, in which chlorophyll-bearing algæ, the chief food of albimanus larvæ, are relatively absent.

Ova of *Anopheles albimanus*, still creamy-white in color, were placed in a breeding tank exposed to the morning sun on December 3; temperature of the water, 28.5° to 30° C. (78° to 82° F.). Of these ova, 5 became larvæ and pupated December 14-15. These pupæ became imagines during the night of 16-17, making the period from ovum to imago about thirteen and one-half days. Under these conditions, they did not get as much sunlight as they would have received outside. Sunlight and the abundance of algæ undoubtedly play a great part in the duration of the period of incubation. It should be added that these 5 mosquitoes, 2 males and 3 females, were placed in a biting jar the morning they emerged (December 17), and that same evening each one of the 3 females bit and drew blood at once when applied to the arm of a patient. In this instance, mosquitoes bit when not more than twenty-four hours old.

Into the same tank were placed 68 larvæ of *A. malefactor* from ova laid December 3; one-third of the ova hatched the morning of December 5; the approximate age of the ova, or the period of incubation, was thirty-six hours. The tank was supplied with algæ, spirogyra, and the water aerated. It was noticed very soon that the malefactor larvæ did not mature as rapidly as the albimanus larvæ did; when specimens of the latter were full grown, the malefactor larvæ were only one-third or one-half grown, and the malefactor larvæ did not pupate until sixteen to twenty days after hatching.

HARDINESS OF *A. ALBIMANUS*.

This mosquito is well fitted for the purpose of transmitting malarial fever. It is the commonest species here at the present time, outnumbering all others, excepting, possibly, *A. pseudopunctipennis*, which latter species is not very hospitable to the malarial parasite.

It breeds in a great variety of locations, besides the customary pools and margins of streams, collections of rain-water, during the dry season it may be found in the stinking water of sewage streams, brackish marshes, running streams, meadows, muddy pools, old crab holes, and in shady malefactor pools, and river margins.

It matures more rapidly than either *A. pseudopunctipennis* or *A. malefactor*.

It outlives *A. pseudopunctipennis* and *A. malefactor*, in confinement at any rate, which is a proof of its ability to persist, for when these species are placed in one breeding jar, *A. malefactor* dies quickly, next follow *A. pseudopunctipennis*, while specimens of *A. albimanus* survive for days longer.

DURATION OF LIFE OF MALES AND FEMALES IN CAPTIVITY.

Male specimens of *A. pseudopunctipennis* which have been kept in breeding jars with females, and supplied with raisins, dates, and water, have lived for eighteen days. Male specimens of *A. albimanus* have lived twelve and one-half days. On the other hand, a virgin specimen of *Stegomyia calopus* has lived for one hundred and ten days.

When virgin anophelines have been given one or two blood meals, two or three days after emerging, they have lived as long as sixteen days. This is in rather striking contrast with *Stegomyia calopus*, which, under similar conditions, lives months to the anophelines' weeks. Several specimens of *S. calopus* virgins still under observation have lived for several weeks, and have oviposited as late as sixty days after emerging, though never in contact with males.

MUSICAL NOTE OF MOSQUITOES.

The characteristic musical note of anophelines is ~~caused by~~ ^{associated with} the vibration of the proboscis, as the following observation indicates:

A specimen of *A. malefactor* was badly wet and sprawled; upon placing her upon a piece of filter paper and touching or approaching her proboscis the latter vibrated visibly, and emitted the characteristic high-pitched note; the wings were at rest, being stuck to the paper. This was verified again and again. Later I picked up a slightly water-sprawled infected mosquito for dissection and held it over a few drops of chloroform; both wings were seen to vibrate rapidly, as in flight, but noiselessly; while holding the mosquito by the last abdominal segment, and touching one wing at its tip the opposite wing would immediately stop vibrating. Upon releasing the wing, both would vibrate noiselessly, as before. The noise of the mosquito is ~~due~~ ^{associated}, then, to the vibration of its proboscis, and the wing vibration is dependently and automatically coordinated.

RELATIVE VALUE OF DIFFERENT FOODS FOR ANOPHELINES.

After trial with bananas, I found that raisins and dates, with water furnished the best food for anophelines in confinement. A greater number of males and females may be kept alive during the few days after emergence if fed with fresh banana, raisins, or dates; but females fed on this diet daily would not bite with alacrity. Mosquitoes fed daily or on alternate days on human blood made better dissections after the digestion and evacuation of their meal than those fed on bananas. The former would be fairly free from yeast and bacteria, and the mid gut and appendages would not disintegrate so rapidly after death. If the mosquitoes were fed alternately on bananas and blood, they would frequently die with an undigested, hard mass of blood in the mid gut, which must have been either impossible to digest or evacuate. The best method of feeding infected mosquitoes would seem to be to feed two or three times on a patient favorable for infection and subsequently with raisins, dates, and water. Then, too, the acid contents of the mid gut, after banana feeding, with its fermentation, may interfere with the infection of mosquitoes by malaria. In experiments Nos. 20 to 28 a patient having at times 67 crescents per 100 leucocytes in his peripheral blood was bitten by four varieties of

mosquitoes, *A. albimanus*, *A. argyritarsis*, *A. pseudopunctipennis*, and *A. malefactor* over a period of thirty-five days. During the intervals between bitings, however, the mosquitoes were fed on bananas and none became infected.

IDENTIFICATION OF LARVÆ.

It must be evident that identifications of anopheline larvæ in the field is of considerable importance, and in this region the malarial-transmitting anophelines can be readily identified by certain anatomical characters. I have made no attempt to determine in detail all the anatomical characteristics of anopheline larvæ of this region; that has been done for some species by Knab. The chief anatomical differentiating larva characters of the common anophelines of this region are these:

A. albimanus or white-footed group:

Larval characters.

Palmate hairs on all abdominal segments and sometimes on postero external angle of the thorax.

Antennæ without a tuft of hairs.

A. pseudopunctipennis group:

Palmate hairs on third, fourth, fifth, sixth, seventh abdominal segments, but none on the first and second. On the latter two, however, there is a rudimentary stalked tuft.

Antennæ without a tuft of hairs.

A. malefactor or spotted-legged group:

No palmate hairs on first and second abdominal segments, but palmate hairs on all remaining segments.

Antennæ with a tuft of hairs.

These characters are very striking and sharply separate the groups, thus separating the malarial transmitting *A. albimanus* group from other varieties. With care it is frequently possible, even in muddy water, from an examination of the indentations of the surface film caused by the palmate hairs, to at once determine the presence or absence of members of the *A. albimanus* group. In the latter group there is no break in the indented film, but in the two former groups there is a well-defined nonindented break in the film, due to the lack of palmate hairs with first and second abdominal segments.

The size of the palmate hairs on the postero lateral angle of the thorax and the presence of these hairs on the thorax, first and second abdominal segments, is subject to some variation. It would seem that the white hind-footed group are undergoing some variation with regard to the size and location of these hairs, and apparently they are becoming rudimentary or vestigial on the thorax and first abdominal segment.

FOOD OF ANOPHELES LARVÆ.

The generally separate and distinct breeding places of *A. malefactor* and *A. albimanus*, for instance, naturally suggest that their food might also be different. Dissection of specimens of *A. pseudopunctipennis* and *A. albimanus* in all instances discloses much green algæ in the intestinal tract, while specimens of *A. malefactor* usually contain much dark brown, unidentified vegetable fibers, brown organic débris, and, conidia resembling those of *Pestalotia trunculata*, *Leptosporum bifurcatum*, *Paramæcia*, etc., *Rotifer vulgaris*. This indicates that *A. albimanus* and *A. pseudopunctipennis* prefer sunny

pools, while *A. malefactor* prefers shady ones, where there is a relative absence of chlorophyll-bearing forms. This is not intended as an absolute statement, because during the dry season, and in certain situations, *A. malefactor* and *A. albimanus* will be found together in the same streams or pools, but it indicates certain different tendencies in the respective species.

Blood feeding necessary for anophelines.—A blood meal seems to be necessary for the development of the ova of anophelines. In experiment No. 40, to determine this point, male and females, *A. albimanus* and *A. pseudopunctipennis* were placed in a breeding jar and fed on vegetable food and water daily, but they received no blood meals. Upon dissection of females as they died, none showed any development of the ovaries.

PARTHENOGENESIS.

If, however, there be given one blood meal, the ova may develop, even in virgins, kept out of contact with males. In the latter instance (with *Stegomyia calopus*) the ova have never developed into larvæ.

Experiment: Virgin anophelines bred out from single isolated pupæ were transferred to one jar entirely out of contact with males. There were three *A. albimanus* and two *A. pseudopunctipennis*. When applied to the arm all the *A. albimanus* drew blood; neither of the *A. pseudopunctipennis* would bite. The following day one *A. pseudopunctipennis* died, but the remaining one, with two *A. albimanus*, bit again upon application to the arm. These were added to another jar of virgin females, *A. albimanus* and *A. pseudopunctipennis*, most of which drew blood readily. Upon dissection none of the *A. pseudopunctipennis* showed any development of the ovaries. One *A. albimanus*, about fourteen days old, contained ova 0.48 mm. long. The single spermathecæ of the mosquitoes were examined, and in no instance contained spermatozoa. The ova would not develop into larvæ in water, and upon microscopic examination were found to contain finely granular food material, but no partly developed larvæ, such as would be seen in fertile ova of this size.

A further experiment with *Stegomyia calopus* indicates that whereas ova may develop in size in the ovaries of unfecundated virgins, they are always sterile, and never contain partly developed larvæ, but granular, undifferentiated protoplasm or food material.

A. November 11; a jar containing five female *Stegomyia calopus* which had been separated individually as pupæ, and always out of contact with males; emerged November 9. They were applied to the arm and three bit and drew blood; they were not as voracious as anophelines, but behaved with the caution and timidity characteristic of *Stegomyia*. After fourteen days these mosquitoes had not oviposited. Two were dissected and the spermathecæ found to be free from spermatozoa, but their ova were well developed, 0.560 mm. in length, 0.184 mm. in width.

B. Bred out 19 virgins, *Stegomyia calopus*, in the same manner, from isolated pupæ. Upon applying to arm, 16 out of 19 bit and apparently drew blood, but there was no sensation of stinging. The next day when applied, only three mosquitoes bit. These may have been the ones that did not bite the day before. Thirty-seven days after the first biting, 8 of these virgins were living; the dead ones

having become water sprawled. Forty black ova were found in the water dish this morning. Forty-one days after the first biting 6 ova were found in the dish. Fifty-four days after the first feeding one dead female was found, and upon dissection contained no ova. Sixty-nine days after the first feeding a dead female was found to contain 20 well developed ova 0.560 mm. in length. Sixty-one days after first feeding 43 black ova were found in the dish, completely developed, 0.720 mm. in length, 0.240 mm. in width. But none of these ova developed later into larvæ. One hundred and ten days after the first feeding the remaining female died.

EFFECT OF SALT AND SEA WATER ON ANOPHELES LARVÆ.

In general, the effect of an irritating, toxic, or otherwise unusual fluid on mosquito larvæ is to hasten pupation. A number of experiments were tried with sea water, salt water, and solutions of the heavy metals, and in most instances, in the more concentrated solutions, when the larvæ were not killed within twenty-four hours they pupated, and occasionally the period of pupation was shortened; so that if, for instance, in a district sea water were used as a larvacide the first effect would be to hasten pupation, and thus increase the number of anophelines in the district, and if later the sea water became diluted by rain several species of malarial transmitting anophelines might breed in it without difficulty; notably, *A. albimanus* and *A. tarsimaculata*. On this account, sea water could not be used with any degree of success as a larvacide for anophelines, except in large quantities and in certain locations.

Chlorine content of natural waters in which mosquito larvæ have been taken and in some instances bred out.

	Per cent of sodium chloride.
<i>A. pseudopunctipennis</i>	0.00165
<i>A. albimanus</i>	1.93
<i>Anopheles</i> (Sp.).....	.65
<i>A. albimanus</i>	1.165
<i>A. albimanus</i>	.255
<i>A. malefactor</i>	.16
<i>A. albimanus</i>	.16
<i>A. malefactor</i>	.00002
<i>A. albimanus</i>	.00125
<i>A. tarsimaculata</i>	{ .16 .21 .63 1.02
<i>A. albimanus</i>	.02
<i>A. pseudopunctipennis</i>	.02
<i>Stegomyia calopus</i>	.26
<i>Culex</i> (Sp.).....	.057
<i>Aedes tæniorynchus</i>	2.200

Sea water taken from Panama Bay contained 3 per cent of sodium chloride, and a sample from Limon Bay (Atlantic) contained 3.17 per cent, so that it will be seen from the foregoing table that some anophelines, under stress of circumstances, may breed in very brackish water.

EXPERIMENTS WITH LARVACIDES.

A number of experiments were carried out for the purpose of obtaining a cheap and efficient preparation for destroying mosquito larvæ. Crude petroleum oil was frequently too viscid to have a spreading power of the highest efficiency. When mixed with crude carbolic acid, however, its spreading powers were increased. From laboratory tests it was determined that crude petroleum for surface use on pools should not be heavier than $-^{\circ}$ Baumé (American standard).

Much of the crude carbolic acid supplied had been found upon analyses to consist chiefly of inert neutral oils with a small proportion, 5 to 10 per cent, of tar acids, and, as this crude acid was used extensively as a disinfectant, experiments were conducted for the purpose of utilizing, if possible, this crude carbolic acid as a disinfectant and larvacide. It was found that crude carbolic acid, having a specific gravity not greater than 0.96 or 0.97, and containing about 20 per cent of phenols or tar acids, when made into a soap, with common resin and an alkali, yielded a product which was an ideal larvacide, having excellent diffusing and toxic powers, and at the same time was a very efficient germicide. It diffused perfectly with water, forming a milky emulsion very destructive to mosquito larvæ, and having a germicidal value of, or greater than, that of pure carbolic acid or a Rideal-Walker coefficient of 1 to 2. In this way a very valuable larvacide and disinfectant, miscible with water, was produced from a very inferior insoluble disinfectant.

The larvacidal powers, when tried with *Culex* and *anopheles* larvæ, varied slightly with the quality of the crude carbolic acid, but an average result is as follows:

- Dilution 1 to 1,000: *Culex* larvæ dead in five minutes.
 Anopheles larvæ, half grown, dead in five minutes.
 Anopheles larvæ, full grown, dead in ten minutes.
- Dilution 1 to 5,000: *Anopheles* larvæ, half and full grown, dead in five minutes.
 Culex larvæ, half grown, dead in three minutes.
- Dilution 1 to 10,000: *Culex* larvæ, half grown, dead in sixty-four minutes.
 Anopheles larvæ, young, dead in fifty-two minutes.
 Anopheles larvæ, full grown, dead in one hundred and thirty-five minutes.
- Dilution 1 to 15,000: Small *Culex* larvæ, dead in thirty-two minutes.
 Anopheles larvæ, full grown, dead in one hundred and twenty-three minutes.

Anopheline larvæ seem to be slightly more resistant than *Culex* larvæ, and all pupæ are more resistant to the effects of the larvacide than larvæ are.

EXPERIMENTS WITH AGENTS DESTRUCTIVE TO VEGETATION, GRASS, AND ALGÆ.

A series of experiments was carried out with the larvacide, caustic soda, arsenic, and copper sulphate, as to the amounts necessary in pools and lagoons to prevent the growth of vegetation and to determine the value of the resulting solutions as larvacides. Bermuda grass in sod was made into artificial ponds in large glass moist jars, and flooded with 0.5 per cent solution of caustic soda, copper sulphate, and sulphuric acid. The sod was well soaked with the chemical solution, but the grass remained vigorous in each instance.

The jars were undisturbed for a period of eighteen days, when a number of *Culex* larvæ were introduced into the solution of the artificial pools. The larvæ were killed within twenty-four hours in the pools containing copper sulphate and sulphuric acid; but those in the pool containing caustic soda remained alive several days. It was concluded from this that none of the above chemicals could be used to advantage in killing gross vegetable matter, such as grasses, and none were of special value as larvacides.

An artificial pool, as above, was flooded with a 0.125 per cent solution of sodium arsenite. All but three or four of the stalks of grass were killed and overgrown with mold, the wilting effect becoming apparent in forty-eight hours. After nine days, when the grass was quite dead, several *Culex* larvæ were introduced into the pool, and were killed after one hour's exposure. The pool was twice flushed out to rid it of arsenic salt, but the grass showed no further signs of life at the end of thirty-five days. It was concluded from this that a 0.125 per cent solution is a valuable agent in destroying gross vegetable forms, such as grass, and the resulting water in the pool remained effective as a larvacide.

The common, green, filamentous algæ, *Spirogyra*, and *Culex* larvæ were introduced into small glass jars containing various high dilutions of copper sulphate and sodium arsenite. The results of two series of experiments showed that copper sulphate in dilutions up to 1 part in 500,000 is inimical to the growth of this algæ. They become grayish-green in color, shrunken, and lose their fresh and crisp appearance. As a larvacide, however, copper sulphate is not destructive in dilutions higher than 1 in 50,000 parts. Sodium arsenite, on the contrary, seems to stimulate the growth of these algæ in all dilutions between 1 in 2,500 and 1 in 25,000,000, the algæ remaining green and vigorous. As a larvacide, *Culex* larvæ were destroyed in sodium arsenite dilutions up to 1 in 100,000. The larvacidal powers of sodium arsenite solutions in contact with green algæ seem to vary within wide limits, depending probably upon the power of the algæ to take the arsenic salt out of solution into its own protoplasm, thus rendering the surrounding solution less larvacidal. It is concluded from this that copper sulphate is more efficient than sodium arsenite as an algacide in high dilutions, but the arsenic salt is a better larvacidal agent. These results are in keeping with our pharmacological knowledge of the effect of copper and arsenic salts in high dilutions on animal and vegetable protoplasm. It would seem, then, that when grass and algæ in pools, without outlets, are to be destroyed, sodium arsenite would be of considerable value for this purpose, and would continue to be efficient until washed or drained out and that copper sulphate is a valuable algacidal agent for the destruction of filamentous algæ, such as *Spirogyra*.

In experiments with the coal-tar larvacide in laboratory tanks and under actual conditions, the coal-tar larvacide was found destructive to grass in dilutions of 10 per cent, the grass turning brown in two or three days and drying in five to six days. When *Spirogyra* was treated with the larvacide, dilutions of 1 to 2,500 were sufficient to kill, while dilutions of 1 to 5,000 and 1 to 10,000 greatly reduced its vigor.

COMPOSITION AND SIZE OF MESH OF WIRE SCREENING.

Two extremely important factors in the use of wire screening for protection against mosquitoes are, first, the size of the mesh and, secondly, the chemical composition of the wire used. In regions where it is only necessary or desirable to protect against anophelines, a No. 16 mesh screening (16 holes to the inch) would answer the purpose; and where, as in this region, it is necessary to protect against some of the smaller varieties, such as *Stegomyia calopus*, a No. 16 mesh would be practically safe, but not absolutely so. The following experiments were conducted to determine the varieties of mosquitoes which would, under stress of circumstances, pass through a No. 16 mesh wire screening. Out of several hundred mosquitoes, 8 common varieties were able to make their escape through a No. 16 mesh wire:

	Number of specimens escaped.
<i>Stegomyia calopus</i> :	
Males.....	10
Females.....	6
<i>Culex cubensis</i> , male.....	1
<i>Culex rejector</i> , male.....	1
<i>Culex extricator</i> , female.....	1
<i>Aedes angustivittatus</i> , female.....	1
<i>Uranotænia lowii</i> , female.....	1

No specimens of *Anopheles albimanus* or *A. pseudopunctipennis* escaped through No. 16 wire-mesh screen, although several hundred were tried. The methods adopted were as follows:

A. A square wooden box, well ventilated, with fine crinoline gauze screening on two sides, and glass on the other two sides, with a central replaceable partition, covered with No. 16 mesh-wire screening, was constructed. Several dozen mosquitoes at a time of the above varieties were liberated on one side of the partition, without food or water, and on the opposite side, close to the screen partition, were placed water, banana, candy, sugar, and raisins as bait. Only three mosquitoes, out of several hundred, of several varieties, passes through the No. 16 mesh partition under the conditions of the experiment. As the space including the mosquitoes was about one-half of a cubic foot in volume, and as there were a few recesses in which the mosquitoes could hide, an electric-light bulb was hung in such a position at night that the mosquitoes would be attracted by it, but this did not favor the passage of mosquitoes through the screen. Tobacco fumes were passed into the mosquito compartment with a rubber-bulb apparatus, and while this excited the mosquitoes, did not cause any of them to escape through the screen. When a person's arm was introduced into the compartment close to the No. 16 mesh-wire partition, it did not induce the mosquitoes to escape through the screening.

B. Next a lantern chimney, covered on one side with fine mesh crinolin gauze, and on the other side with a metal collar, holding in place a piece of the No. 16 mesh-wire screening, was partly filled with various mosquitoes and placed near the same bait, as before, under a large glass bell jar. Eighteen mosquitoes escaped from the chimney through the No. 16 mesh screening into the surrounding jar. The closer quarters and the absence of resting places in the chimney evidently favored the escape of mosquitoes through the wire screen-

ing. On one occasion, by passing a gust of air through the lantern chimney jar, a male *Culex* was helped through and escaped.

The conditions in the experiments were all rigid and more extreme than those under actual conditions where mosquitoes are trying to enter a screened house from the open.

The chemical composition of various screening material used was investigated by Dr. R. W. Nauss, formerly of this laboratory. Screening of excellent quality was compared with that which had deteriorated more or less rapidly and analyses of screens and their incrustations made to determine the factors concerned in its corrosion. In the investigation considerable attention was paid to the analyses of the efflorescence or incrustation formed on the screening for the determination of the constituents involved in the corrosion. The specimens presenting the highest degrees of deterioration furnished the largest amounts of incrustation. The deterioration of the screening is largely due to the presence of iron in the brass alloy, plus the influence of a hot, moist atmosphere.

Observations continued over a period of four years on screening made of copper and zinc with a composition nearly:

Copper.....	84.92	89.94	84.83	88.59	95.85
Zinc.....			14.90		4.15
Iron.....			0.06	.04	0.0

showed that these resist the corroding actions of a hot, moist climate much better than screening made of brass with an average composition of: Copper, 65; zinc, 34; iron, ± 1 ; and it is concluded that screening intended for use in the Tropics, exposed to heat and moisture, should have a high copper content, higher than brass, and be as free as possible from the presence of iron.

VALUE OF DAILY COLLECTION AND DESTRUCTION OF LIVE MOSQUITOES CAUGHT IN BARRACKS AND QUARTERS.

Abundant material was received for observations on the value of this practice. The daily catch of mosquitoes from barracks of various districts would be sent alive to the laboratory. The mosquitoes were transferred to breeding jars and fed on dates or raisins until their intestinal tracts were free from blood. They were then killed and examined for zygotes or sporozoites. The species examined were: *A. albimanus*, *A. tarsimaculata*, *A. argyritarsis*, *A. malefactor*, and *A. pseudopunctipennis*. Quite a number of specimens of *Culices* were also examined at this time. It is noteworthy, and speaks well for the practice of these daily killings, that but one naturally infected anopheline was found, and that one a specimen of *A. argyritarsis*. After the first 43 negative dissections, no record was kept of the total number examined, but it was in the neighborhood of 500.

EFFECT OF QUININE ON THE MALARIAL PARASITE IN THE (a) MOSQUITO AND (b) MAN.

A. Nearly all the infecting experiments were conducted on patients who were receiving the routine ward treatment of quinine, grains 10, t. i. d., in solution, so that apparently quinine in these quantities has no destructive or inhibitive effect on the parasites in the mosquito because the zygotes go on to maturity and sporozoites appear in the salivary glands in from nine to eleven and one-half days.

One experiment should be mentioned, however, because the patient received no quinine for several days before the mosquitoes became infected and none during the experiment, so that the mosquitoes never received any quinine. One *A. albimanus* contained the rather large number of 168 zygotes upon dissection. It should be mentioned as well that from this patient two *A. pseudopunctipennis* became infected. It may be that quinine has a slight inhibitory effect on the parasite in the mosquito's mid gut.

B. The following tables show the gradual but steady decrease in the number of gametes in the peripheral blood during the administration of quinine, grains 10, t. i. d., in solution, and one table shows the effect of withholding quinine.

The differential leucocyte counts are tabulated as well, and in these the relative increase in mononuclear elements—lymphocytes intermediate and large mononuclear cells—the latter being the chief circulating phagocytic cell in malaria.

As indicative of blood regeneration and the secondary effects on the hemopoietic organs, it is interesting to note the increase in the eosinophiles.

Chart 51499.

	De- cem- ber 30.	De- cem- ber 31.	Janu- ary 2.	Janu- ary 5.	Janu- ary 6.	Janu- ary 8.	Janu- ary 13.	Janu- ary 15.	Janu- ary 23.	Febru- ary 1.
Ploymorphonucle.....	68	65	75	81	65	61. 5	65	51	53	66
Large lymphocytes.....	4	16	6	5	6	13. 5	13	11	9. 5	2. 5
Small lymphocytes.....	20	13	13	11	20	16. 5	11	14	18	11. 5
Intermediates.....								8	4	7. 5
Eosinophiles.....	7	5	5	2	8	7	11	14	14. 5	12. 5
Mast.....	1	1	1	1	1	1. 5	0	2	1	0
Crescents.....	67	42	76	46	40	15. 5	9	5	0. 5	0

The table given above shows the rate of diminution in the number of gametes (crescents) by the administration of quinine, grains 10, t. i. d., in a Spaniard, 60 years of age, on Isthmus twenty months, whose blood contained numerous crescents, but no young forms, and whose temperature was 97° F. continuously. It also shows the degree of perturbation in the proportion of leucocytes-eosinophilia, of increase in mast cells, very slight lymphocytosis and polymorphonuclear decrease.

Compare this with the following: Case 48987 of estivo-autumnal malarial fever, from whom quinine was withheld for twenty-four days: Spaniard, on the Isthmus three months, temperature normal on admission:

1908.	Novem- ber 11.	Novem- ber 24.	Novem- ber 27.	Novem- ber 30.	Decem- ber 4.
Polymorphonuclear.....	86	53	62	42	44
Large lymphocytes.....	2	3	22	11	16
Small lymphocytes.....	9	39	14	46	38
Eosinophiles.....	2	3	0	1	2
Mast.....	1	0	0	0	0
Transitional.....	0	2	2	0	0
Crescents.....	16	27	12	8	20

In this case the continuance of the gametes in the peripheral blood is striking. The polymorphonuclear decrease, and the lymphocytosis

should be noted. This patient received no quinine during the period between November 11 and December 6. Young estivo-autumnal forms were always present in his blood with gametes. His temperature was irregularly perturbed and irregularly quotidian in character.

53937. Spaniard, on the Isthmus twenty months, temperature normal, blood contained on admission many crescents, but no estivo-autumnal merozoites; quinine, grains 10, t. i. d., with Fowler's solution, gtts., 5.

Chart No. 53937.

	Feb- ruary 5.	Feb- ruary 6.	Feb- ruary 8.	Feb- ruary 9.	Feb- ruary 11.	Feb- ruary 14.	Feb- ruary 15.	Feb- ruary 16.	Feb- ruary 17.	Feb- ruary 19.
Polymorphonuclear.....	69	41	41	42	40	52	33	40	39	52.5
Large lymphocytes.....	8	33	20	11	19	11	20	18	23	7.5
Small lymphocytes.....	18	17	23	34	28	22.5	24	25	23	23
Intermediates.....	3	8	10	8	5	10.5	6	14	10	7
Mast.....	0	0	0	0	1	.5	.3	0	2	.5
Eosinophiles.....	2	1	6	5	7	3.5	9	3	3	9.5
Crescents.....	92	87	61	48	20	3.5	4	3	2	.5

This table illustrates, as in the preceding one, the steady disappearance of crescents under quinine, grains 10, t. i. d., also the perturbation in the proportions of leucocytes.

It should be said that the specimens of blood were always taken at 4.30 or 8.30 p. m., or about four hours after a meal.

53742. Spaniard, sixteen months on the Isthmus, blood, estivo-autumnal rings, crescents, ovoids. Spleen enlarged at umbilicus. February 1, quinine, grains 10, t. i. d., February 6, Fowler's solution, gtt. 5 t. i. d.

No. 53742.

	Febru- ary 1.	Febru- ary 2.	Febru- ary 3.	Febru- ary 5.	Febru- ary 6.	Febru- ary 8.	Febru- ary 9.	Febru- ary 11.
Polymorphonuclear.....	56	48	36	44	56	42	43	42
Large lymphocytes.....	17	22	16	15	12	18	25	27
Small lymphocytes.....	15	21	27	24	8	18	14	12
Intermediate.....	8	4	3	8	10	12	8	9
Eosinophiles.....	3	5	15	7	13	9	10	9
Mast.....	1	0	2	1	1	1	0	1
Pigmented, large.....			1	1				
Crescents.....	29	22	29	14	10	5	5	2

NOTE.—Blood, February 2, 6,500 leucocytes per 1 cm.; February 5, contained one phagocyted gamete.

The large mononuclear increase is striking.

M. L., Spaniard, estivo-autumnal malaria, returned and died. Autopsy, March 28, 1909. Quinine, grains 10, t. i. d.

	Decem- ber 26.	Decem- ber 27.	Decem- ber 28.	Decem- ber 30.	Decem- ber 31.	Janu- ary 2.	Janu- ary 13.
Polymorphonuclear.....	48	65	60	64	70	65	45
Large lymphocytes.....	13	6	5	9	3	12	5
Small lymphocytes.....	34	25	32	14	22	14	34
Eosinophiles.....	4	4	3	12	5	8	16
Mast.....				1		1	
Pigmented, large.....	1						
Crescents.....	9	5	5	4	1	0	0

NOTE.—Poikilocytosis and basophilia of red blood corpuscles on January 13.

The following two records are taken from cases of tertian malaria receiving quinine, grains 10, t. i. d.:

No. 51431.

	Decem- ber 26.	Decem- ber 27.	Decem- ber 28.	Decem- ber 30.
Polymorphonuclear.....	78	71	49	64
Large lymphocytes.....	8	6	27	8
Small lymphocytes.....	13	22	24	28
Eosinophiles.....	1	1	0	
Gametes.....	3	8-10	4	0

No. 51147.

	Decem- ber 21.	Decem- ber 23.	Decem- ber 26.
Polymorphonuclear.....	69	18	48
Large lymphocytes.....	7	39	12
Small lymphocytes.....	23	36	30
Mast.....	1		
Eosinophiles.....	0	7	10
Gametes.....	3	1	0

Quinine discontinued after an initial dose.

No. 50792.

	Decem- ber 14.	Decem- ber 15.	Decem- ber 17.	Decem- ber 20.
Polymorphonuclear.....	64	48	17	23.5
Large lymphocytes.....	19	17	28	24
Small lymphocytes.....	7	15	45	41
Eosinophiles.....	10	19	8	10
Mast.....	0	1	1	.5
Pigmented, large.....	0	0	1	.5
Gametes.....	(?)	(?)	0	0

Quinine discontinued after an initial dose. Returned January 11 with estivo-autumnal malaria—7 crescents per 100 leucocytes.

Hospital No. 50782.

	Decem- ber 14.	Decem- ber 15.	Decem- ber 17.	Decem- ber 20.	Janu- ary 13.
Polymorphonuclear.....	47	40	20	47	42
Large lymphocytes.....	34	34	39	13	11
Small lymphocytes.....	16	26	37	37	42
Eosinophiles.....	3	0	4	2	4
Mast.....	0	0	0	1	
Gametes ^a	0	0	0	0	

^a None of 7 *A. albimanus* became infected from this case, confirming the absence of gametes films.

In each of the above tertian cases the rapid polymorphonuclear decrease and the equally rapid mononuclear increase should be noted.

The effect of quinine administration, then, is to make the gametes gradually disappear from the peripheral blood by the destruction

of the young forms, the gametes being phagocyted by splenic and hepatic endothelium. It is concluded that quinine, grains 10 t. i. d., in solution will gradually reduce the sexual form of the parasite in man to a noninfective minimum in from a few days to a few weeks, depending on the severity of the infection.

In tertian malarial fever gametes disappear from the peripheral blood within two or three days under quinine treatment, and generally disappear even when quinine is withheld, if the patient is at rest. There are never as many gametes in the peripheral blood in tertian as in estivo-autumnal malaria. As a consequence one never finds as many tertian zygotes as estivo-autumnal zygotes in infected mosquitoes.

LATENT MALARIA AMONG LABORERS AND THEIR FAMILIES.

This subject was taken up in March, 1909, during the dry season when very few mosquitoes were breeding, so that the number of cases of malaria resulted more frequently from recrudescence than new infections. In two locations, for instance, Spanish barracks at Ancon and Spanish barracks and negro quarters at Cucaracha, there were certainly no mosquitoes breeding and none taken in quarters—good indications that there were no cases of new infections. The blood specimens were taken at one time between 5 and 6 p. m. on one day when the men were coming out from supper. Two hundred and thirty-seven specimens out of a possible 269 laborers were taken. Few of the men were away from camp and a few rebelled—refused to permit the taking of specimens.

The result of the examination is given:

- 1 half-grown tertian.
- 1 pigmented mononuclear.
- 1 ring form.
- 1 pigmented mononuclear—1 ring; discharged from hospital nine days ago.
- 2 half-grown tertians.
- 1 pigmented mononuclear.
- 1 crescent; several pigmented mononuclear.
- 1 large mononuclear increase.
- 1 tertian, all ages.
- 1 blood negative; entered hospital; few days later with tertian malaria; tertian rings all negative.
- 1 tertian ring.
- 1 pigmented lymphocyte; one-third grown tertian; 1 presegmenter.
- 2 tertians, three-fourths grown.
- 1 tertian, one-half grown.
- 1 crescent.
- 1 crescent.
- 1 tertian gamete.
- 2 one-half grown tertians.
- 1 pigmented mononuclear.
- Several three-fourths grown tertians.
- Several one-half grown tertians.
- Several three-fourths grown tertians.
- Several one-half grown tertians.
- 2 crescents.
- Tertian one-half grown, full grown, and gametes.
- 2 crescents.
- Normal; entered hospital a few days later with tertian malaria.
- Full-grown tertians.
- 2 young tertians.

These were all Spanish laborers who had been at work and were working every day. Their check numbers were taken at the time, and in this way it was ascertained which of them entered the hospital within the ensuing few days. It was rather surprising to find that those with positive blood did not enter, while two with negative blood at the time developed symptoms and entered the hospital with tertian malaria.

At Cucaracha, about 2 miles from Culebra, a number of Spanish laborers were in the habit of eating at an unscreened restaurant on the hill above the camp and of sitting around after dark.

To determine the infectivity of the employees at the restaurant, their blood was examined with the following result:

Proprietor, 1 large ring; one-half grown tertian.

Wife, negative.

Daughter, 1 tertian ring.

Waiter, 1 small estivo-autumnal ring.

Waiter, 1 crescent.

At the time mosquitoes were not breeding at this locality, but during the rainy season it is not unlikely that laborers become infected at this place.

About a mile back in the bush the blood of a native family was examined, for on Sundays and holidays the Spanish laborers were in the habit of picknicking near by. A bush boy, girl, two women, boy, and two men—seven individuals, including three young persons—were examined, yet their blood was entirely negative.

The Spanish laborers at Cucaracha lived in screened quarters, and of 53 found at home one Sunday morning the blood of 7 contained malarial parasites, 6 tertians, and 1 probably estivo-autumnal—13.2 per cent.

Near by were the quarters of married Spanish laborers and West Indian negro families in houses that had only recently been screened, so that they had been unprotected during the preceding wet season.

Of 6 Spanish children examined, 4 were infected:

First. Several large rings, probably tertian; one red blood cell containing two rings.

Second. Negative.

Third. Negative.

Fourth. One tertian ring.

Fifth. One tertian ring.

Sixth. Tertianen all ages.

Spanish mother. Negative.

Spanish father. Two tertian rings.

Of 13 adult individuals living in recently screened West Indian barracks for married persons, 6 or 46 per cent, were infected, all tertian infections.

From this examination of laborers at work, 8 to 13 per cent of latent malaria was met with, chiefly of the tertian variety in the proportion of about 4 of tertian to 1 of estivo-autumnal. Among children and adults living in unscreened or recently screened barracks the percentage of latent malaria was, as previously indicated, much higher.

It is this latent, untreated malaria in every tropical community which contributes largely to the preservation of the malarial parasite and to the infection of anophelines when, after the onset of the rainy season, mosquitoes have begun to breed in numbers.

VALUE OF THE SPLENIC INDEX IN DETERMINING THE AMOUNT OF
MALARIA IN A COMMUNITY.

The degree of splenic enlargement in a malarial region seems to depend on the following factors:

- (a) Amount of blood destruction or loss.
- (b) Duration of the blood destruction.
- (c) Ability of the hemopoietic organs to regenerate red blood cells.
- (d) Degree of reaction to the infection as in such infections as relapsing fever, where there is at first not a high degree of blood destruction or, at any rate, the splenic enlargement is so rapid and the red count so little, decreased, perhaps, that the splenic enlargement is a measure of toxemia rather than blood destruction. When there is much blood destruction or depletion and the blood-forming organs are not passive, then the splenic enlargement will be considerable.

In this region there is a source of splenic enlargement which is confused with that of malaria. I refer to uncinariasis. Some of the largest spleens encountered here are undoubtedly those secondary to uncinariasis anæmia.

Data bearing on this point were obtained by an examination of native school children at old Porto Bello.

Out of 30 boys of the village, 6 to 10 years of age, 15 with enlarged spleens were examined for malarial parasites and all with negative result. There was, however, a decided œsinophilia in each instance. As this was associated with protuberant abdomen, pallor of conjunctivæ, I regard the condition as that of uncinariasis and not malaria.

So that in a region infected with uncinariasis, as well as malaria, the splenic index is an index of the former as well as the latter.

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